IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Peter M. Goodwin et al.	Docket No.:	S-94,652
Serial No.:	09/862,855	Examiner:	Strzelecka
Filed :	5/21/2001	Art Unit:	1637
For :	RAPID HAPLOTYPING BY SINGLE MOLECULE DETECTION		

DECLARATION OF PETER M. GOODWIN, JAMES H. WERNER, RICHARD A. KELLER AND HONG CAI UNDER 37 CFR 1.131

We, Peter M. Goodwin, James H. Werner, Richard A. Keller and Hong Cai, hereby state and declare:

1. We are the inventors of the invention described and claimed in the above-referenced patent application (the "subject invention").

2. On or before March 13, 2000, we submitted an invention disclosure for the subject invention to patent counsel for the Regents of the University of California at Los Alamos National Laboratory.

3. A true copy of the invention disclosure is attached hereto as Exhibit A.

4. The invention disclosure describes a complete conception of the subject invention.

5. In particular, the last paragraph on page 2 of the disclosure describes the method of the invention as follows:

> *We have developed the capability to detect and identify single fluorescent molecules. Our approach to haploidtyping is to label the SNP probes with different color fluorescent molecules, denature the DNA (separate the strands), hybridize the probes, and screen for single chromosomes (haploids) containing both of the SNP probes (this is a two color analysis). Our strategy is shown in Figure 2.*

6. The above-referenced paragraph also describes how the method is applied to the determination of haploidtypes of four different types of individuals using two sets of SNP-specific probes.

7. Figure 2 on page 3 of the disclosure presents a schematic representation of the two color single molecule haploidtyping method of the subject invention.

8. The subject is further described in terms of its advantages over prior art methods in, for example, the second full paragraph on page 5 of the disclosure, which advantages include the absence of nucleic acid amplification in the method.

9. A provisional patent application describing and claiming the subject invention was diligently pursued and filed approximately two months after we submitted the disclosure (US 06/206,512, filed May 22, 2000). The subject application claims priority to this provisional patent application.

10. All statements made herein of our own knowledge are true and all statements made on information and belief are believed to be true, and further, these statements were made with the knowledge that willful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Peter M. Goodwin	10/16/06 Date
James H. Werner	10/16/06 Date
Richard A. Keller	10/16/06 Date
Hong Cai	10/16/06 Date

EXHIBIT A

Los Alamos NATIONAL LABORATORY

MAR 1 3 Invention Disclosure

LAD Number: 3000-030
(IBD use only)

Instructions: When completed, submit to Industrial Business Development IPM Copyright Team, MS C334, Phone 5-7380, Fax 5-0154.

1. Title of Invention
Rapid Haploidtyping by Multicolor Single Molecule Detection

2. Invention Description (please attach)

3. Background Search (If you have done a background search for related patents and publications, please attach.)

4. Funding
Did this invention result from some type of collaborative arrangement (CRADA, FIA, etc.)?
☐ Yes ☒ No If yes, please list: Agreement Number ____ Company Name

Invention Development Program and B&R Codes				
	Program Code(s)	RAR8/F159		
	Corresponding B&R Code(s)	?		
	Sponsor(s)/DOE Program(s)	NIH/DOE		

5. Conception and Public Disclosure (Accurate data is essential as prior disclosure may affect the possibility for obtaining patent rights.)

	Date	References/Comments Please include names of periodicals/journals. (Use separate sheet if necessary)
(a) Date of conception of technology. Has this date been documented? If so, where?	Nov 9th,1999	Hong Cai's notebook #3 page118, page, 120, TA43 B150, LANL
(b) First publication containing any description of the technology. (Include thesis and the date submitted.)	NIH proposal, Jan 7th/2000	copy attached
(c) First public oral disclosure of the technology.	none	none
(d) If unpublished and undisclosed, provide the anticipated publication or oral disclosure date and any submissions made for potential publication.	04/00	LDRD proposal

6. Is the invention operable or in use? ☐ Yes ☒ No If yes, give date of operation

7. Attach list of any commercial entities that may be interested in this invention. Provide as much detail as possible.

8. Lab notebooks where invention was first recorded (give number, pages, location):
Nov 9th, 1999, Hong Cai's notebook #3 page118, page, 120, TA43 B150, LANL

9. Co-Inventors: Note that the employee Z number and country of citizenship are required. If there are more than two inventors, attach additional form.

(a) Printed Name Hong Cai	Phone 505-665-2092	Mail Stop M888	Signature	Date
Home Address 1997 Cumbres Patio, Los Alamos, NM 87545		Z Number 117872	Division & Group BN-2	Country of Citizenship China
(b) Printed Name Peter M. Goodwin	Phone 505-665-2092	Mail Stop M888	Signature	Date 3/10/00
Home Address 4259 Trinity Drive, Los Alamos, NM 87544		Z Number 110726	Division & Group BN-2	Country of Citizenship USA

10 Technology disclosed to and understood by non-inventor witness
Printed Name and Title Angela A. Arias, Postdoc Fellow | Signature | Date 3-10-00

11. Line Manager(s) Approval(s)
I have reviewed the disclosed technology and concur that the technology is accurately represented above and recommend that the UC evaluate the technology for patent protection.

Printed Name and Title Basil Swanson, resource manager	Signature	Date 3-13-00
Printed Name and Title Jill Trewhella, B Division director	Signature	Date 1-13-00

Form 1712 (1/00)

Attachment: Haploidtyping by Multicolor Single Molecular Detection

2. Description:

(a)General Purpose: What does the invention do?

This invention is a single molecule approach to the biological haploidtyping problem, which is important in disease association studies and clinical diagnosis. The current methods of haploidtyping are very complicated, slow and expensive. Our invention will allow sensitive and rapid haploidtyping of small amounts of un-amplified genomic DNA samples.

(b)Technical description: How did you create the invention? How do you use it? We have combined our expertise in genotyping and single molecule fluorescence detection to invent a new method to do haploidtyping on single human chromosomes. We have known the limitations of conventional haploidtyping for a long time, and we recently conceived the idea of the SMD (single molecule detection) haploidtyping. We intend to use it to attract NIH funding. Meanwhile we will obtain more data to validate our new method. We believe that our invention will greatly facilitate the progress of disease association studies as well as clinical disease diagnosis. Here is a brief description of our invention (See attached NIH proposal for a detailed explanation).

Cells contain DNA. DNA is a long linear polymer containing an ordered string of the nucleic acids (nucleotides) adenine (A), cytosine (C), guanine (G), and thymine (T). The DNA is packaged into 23 human chromosome pairs in somatic cells (i.e. body cells). Each chromosome pair contains two individual chromosomes; one chromosome is from the father and the other chromosome is from the mother. The individual chromosomes are called haploids. There are genetic diseases associated with single nucleotide variations [single nucleotide polymorphism (SNP)] in the nucleic acid sequence of these haploid chromosomes. A particular set of SNPs or DNA sequence information on an individual haploid defines the specific **haploidtype** of that haploid chromosome and the process to determine a haploidtype is **haploidtyping** (or haploid genotyping to SNP targets). Haploidtypes are very important to the discovery of disease-associated (or disease-causing) genes and clinical disease diagnosis. For an example, a genetic disease-causing mutation often co-segregates with a particular set of SNP markers on a haploid chromosome (e.g. the copy inherited from one parent). The discovery of a disease mutation depends upon the ability to associate the specific SNP haploidtype with a disease, and then identify the mutation by sequencing the genes close to the related SNP markers. Haploidtyping is also crucial for clinical diagnostics. Examples include bone marrow transplantation donor screening, drug dose determination based on the individual genetic profile and individual responsiveness to chemotherapy. SNPs are typically detected by hybridizing probes complementary to the mutated SNP targets (bulk genotyping). Since there are two haploid copies in human cells (except sperm and egg cells that only contain one set of chromosomes), conventional bulk sequencing measurements indicate the presence of all the sequence identities of SNPs (genotypes) but don't show the location of SNPs on the two different haploids (i.e. they don't give the haploidtypes). As illustrated in Figure 1, genotypes resulted from bulk sequencing may not provide the useful information in clinical diagnosis and drug response information.

Importance of haploidtypes

Individual	Two haploidatypes of an individual		Genotype of an individual	Phenotype	genetic cause
Wild Type two normal SNPs on each chromosome	A	B	A/A-B/B	normal	two good copies
	A	B			
Two mutant SNPs separated on two chromosomes	A	b	A/b-B/b	abnormal	two bad copies
	a	B			
Two mutant SNPs on one chromosome, two normal SNPs on the other chromosome	a	b	A/b-B/b	normal or a little abnormal	one good copy & one bad copy
	A	B			
Mutant two mutant SNPs on each chromosome	a	b	a/a-b/b	abnormal	two bad copies
	a	b			

Figure 1. The importance of haploidtypes. At two target SNP locations in a disease-associated gene, the SNPs associated with a wild type cell (normal cell) are A and B, the SNPs associated with a disease cell are a and b. In principle, there can be four nucleic acid bases possibilities (G, A, T or C) at a given SNP. However, human SNPs are typically bi-allelic which means only two base types are found for a given SNP site, e.g. it's either G or C for a target SNP and A or C for another SNP. Genotype refers to the SNP identities (nucleic acid sequences) at a SNP location. Phenotype here refers to the clinical outcome of an individual.

In general, genotyping does not identify the haploidtype. As illustrated in Figure 1, the genotype A/b-B/b obtained from bulk measurement can come from haploidtypes, (A-b and a-B), or (a-b and A-B) that give very different clinical diagnosis outcomes; (A-b and a-B) is much more disease-causing. Therefore, haploidtyping is very important in clinical disease diagnosis, drug response prediction and treatment.

We have developed the capability to detect and identify single fluorescent molecules. Our approach to haploidtyping is to label the SNP probes with different color fluorescent molecules, denature the DNA (separate the strands), hybridize the probes, and screen for single chromosomes (haploids) containing both of the SNP probes (this is a two color analysis). Our strategy is shown in Figure 2. To determine the haploidtypes of four different types of individuals (left column), two sets of SNP-specific probes targeted to two target SNPs will be used determine the location of the SNPs on each haploid; one set is against normal SNP markers, the other set is against the disease-associated SNP markers. By performing the two color correlation analysis of single haploid chromosomes with two sets of SNP markers, the two SNPs on each haploid chromosome (haploid genotyping or haploidtyping) from an individual can be determined (see Result Column in Figure 2).

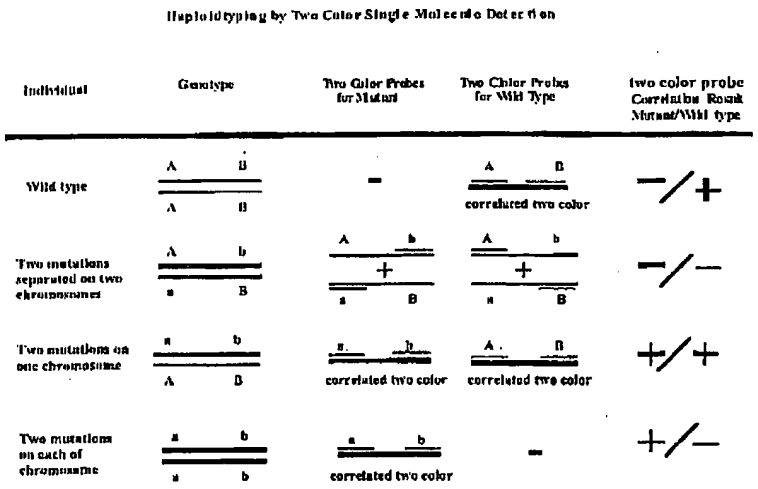

Figure 2. Haploidtyping by two color single molecule detection. At two target SNP locations, the SNPs associated with a wild type cell (normal cell) are A and B, the SNPs associated with a disease cell are a and b. Genotype refers to the SNP identity (nucleic acid sequences) at a SNP location. Black and grey bars are the SNP-specific oligomer probes with different two fluorescent labels that are hybridized to the SNP sites. In the result column, + is a positive two color correlation result and – is a negative two color correlation result. We can distinguish all haploidtypes using this approach.

(c)Advantages and improvements over existing technologies: Describe existing methods, device or materials:

Limitation of Current Haploidtyping Technologies. Currently, haploidtyping is done by bulk genotyping, sperm typing or molecular cloning. As discussed below, all three methods have serious drawbacks that limits their use in disease association studies and clinical diagnostics.

Bulk genotyping: Haploidtypes deduced from genotypes obtained from a bulk sequencing of the mixture of two haploids in cells is typically done as the follows: A region of DNA containing SNP targets is PCR amplified, the nucleic acid sequence at the SNP site is sequenced to determine the genotype of the SNPs, and haploidtypes are deduced from the homozygous individuals (identical sequences on both chromosomes). Since the genotype is based on a bulk measurement of a mixture of both chromosomes, this genotyping approach has serious limitations for multiple SNP marker analysis. The problem is illustrated by a two SNP marker system shown in Figure 2.

A. For any 2-SNP system, there are 9 possible genotypes.

1. A/a-B/B	2. A/a-B/b	3. A/a-b/b
4. A/A-B/B	5. A/A-B/b	6. A/A-b/b
7. a/a-B/B	8. a/a-B/b	9. a/a-b/b

B. For the genotype result, A/a-b/B, there are 4 possible haploidtypes and two possible combinations for an individual.

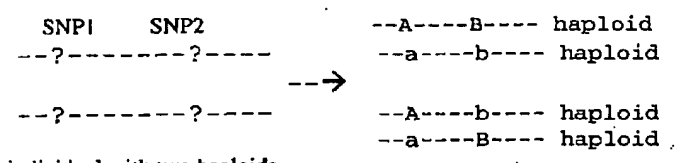

Figure 3. The difficulty of inferring haploidtypes from a genotype. A. On a 2-SNP system, there are 9 possible genotype results and 16 possible haploidtypes (not shown). B. On a 2-SNP system, there are four possible haploidtypes giving the same genotype A/a-B/b. A and a are two bi-allelic forms of the SNP1 target. B and b are two bi-allelic forms of the SNP2 target. Only the bottom pair is disease-causing.

As illustrated in Figure 3. for any two SNP system, there are 9 possible genotypes (Figure 3A) and 16 possible haploidtypes. There are four haploidtype possibilities to give the genotype A/a-B/b. It is estimated 1000 gene targets with an average of 5 SNPs on each gene will be used in the future disease association studies. In that case, one gene with 5-SNP markers will have 243 possible genotypes and 528 possible haploidtypes. As the number of SNP makers grows larger with multiple genes, the identification of the haploidtype from the genotype is increasingly difficult since the number of possible haploidtypes increases rapidly, and the probability of finding a homozygous individual decreases sharply.

Single sperm measurement. DNA containing target SNPs from individual sperm cells is PCR amplified and genotyped. Multiple sperm cells (at least 3-5) from an individual are typed in order to have enough statistical confidence to reveal the two haploidtypes. In principle, this sorting approach could be applied to chromosomes. However, this technique is complicated, and so far, has been successful in only a few research labs.

Molecular cloning method. DNA (or cDNA) fragments containing target SNPs are cloned into a vector, transformed into a bacterial host and amplified as the host cells propagate. Haploid DNA from individual chromosomes is picked and PCR amplified. Genotyping is performed. For each individual, multiple colonies are needed to obtain two haploidtypes from an individual. This method has been used by many laboratories,

but is very labor-intensive, time-consuming and can be difficult to perform in some cases. Researchers are forced to use it because there are no easy alternatives.

In summary, there is no easy way to determine a haploidtype currently except for the sex chromosomes (sex chromosomes X and Y have large sequence differences allowing specific PCR amplification of one sex haploid). All of the above methods require DNA amplification by PCR or cloning. They are slow, labor intensive and expensive to perform, and not well suited for a large-scale association studies, or routine clinical diagnostics.

Advantages of the Single Molecule Approach over Current Approaches. The proposed single molecule haploidtyping approach has a number of advantages over current haploidtyping methods. These are: (1) Easy interpretation and fewer experiments. Our approach is a direct haploid genotyping method. No complicated analysis is needed to rule out other haploidtype combinations. Unlike other haploidtyping methods that require multiple sperms, single clones or experiments, our approach requires fewer experiments for each SNP pair to determine the two haploidtypes of an individual. (2) Ability to type large regions [~200 kilobase(kb) or more] in a single assay. Since the typing is directly performed on the chromosome, the length of the chromosome fragment that be can be typed is limited, by mechanical shearing of the genomic DNA during handling, to ~200 kb. Using a SNP as a reference marker, the neighboring SNPs within 400 kb (200 kb of the left and 200kb of the right of the reference marker) can be haploidtyped. By choosing a different reference marker for every 400 kb, the whole chromosome can be haploidtyped by sequentially typing every SNP pair that contains one target SNP and one reference SNP. This will be significantly simpler than PCR-based typing, which is limited to a range of a few kilobases. (3) High sensitivity. The assay will require only 20 ng of genomic DNA, about the same amount needed for a typical PCR reaction. No amplification of sample DNA is required prior to analysis. This sensitivity is suitable for the analysis of small amounts of tissue samples. (4) High throughput. The confocal microscope arrangement used for fluorescence detection can easily be adapted to a sample droplet array format for high throughput analysis.

(d)Applications: What are the potential uses of the invention?

This invention will provide a powerful tool for the studies of the association between phenotypic behavior and its genetic causes. It will ultimately allows the association study to identify a particular set of the genes associated with a disease using genetic markers, such as SNPs. It is anticipated that the association study will be a major component of the next phase of the human genome project and drug industry.. This tool will also be extremely useful for routine diagnostic analysis in clinical labs. The biochemistry approach in this invention is easy and straightforward, and the device built according to this invention will be a compact bench-top automated unit for the routine clinical usage.

(e)Issues and concerns: Is it sensitive, unclassified material?

No.

No.

(f)Applicable documents; Are there any manuscripts, drafts, or articles that pertain to the invention?

A NIH proposal sent on Jan 7/00 (attached with this form)

(g)Commercial applications:
Commercial applications: disease-associated gene discovery, drug development and discovery, diagnostic device in clinical settings.

3. Background Search: We have conducted a background search, there was nothing that comes close to our invention for haploidtyping.

7. Potential licensees:
-Any large pharmaceutical company doing drug discovery: Such as Merck, DuPont, SmithKline, Amgen, and etc.
-Medical Instrumentation: HP, Pharmacia, Bio-Red, Becton Dickinson, PE-Biosytem and etc.

9. Co-inventors continued:

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